USDA ARS National Animal Germplasm Program

Goat Semen Collection, Transportation, Processing and Cryopreservation Protocol

Semen collection and processing:

Collect semen from sexually mature bucks via an artificial vagina or electroejaculation. The artificial vagina is the preferred method with bucks.

Inspect sample to ensure it is free of urine and other contaminants.

Determine the sperm concentration and motility using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 5 fields of analysis and 500 sperm) or microscopy and a hemocytometer.

Add antibiotics per the National Association of Animal Breeders (NAAB) Certified Semen Services standards (https://www.naab-css.org/). The antibiotics are added to the neat semen and both cryopreservation media. Consult the NAAB website for the timely information regarding the appropriate antibiotics and dosages.

Dilute the samples in a 15 or 50 mL tube to 400 x 10^6 sperm/mL with 37 °C Tris-egg yolk glycerol medium (TEYG; see recipe below).

Optional seminal plasma removal: If coagulation is a concern, which may be the situation with bucks that are known to react with egg yolk, then the semen sample can be washed to remove seminal plasma. This is accomplished by diluting the sample 4 to 5 times with TEYG medium *that is devoid of egg yolk and glycerol*. The diluted sample is centrifuged (800 x g for 10 min) and the sperm concentration is determined. The sample is then diluted with TEYG and processed for cryopreservation as described previously.

If the samples will be transported overnight then, after dilution with TEYG, they are placed in a 37 °C water jacket and cooled to 5 °C over 2 hours in a refrigerator, packaged in an Impact Shipper (see Transportation section below) and shipped to the National Animal Germplasm Program laboratory. Samples can be collected and held for 24 hours at 5 ºC prior to cryopreservation using this diluent because of the low egg yolk concentration (Mook and Wildeus, 2008). If coagulation is a concern remove the seminal plasma as described previously.

-OR-

If samples will not be held for an extended period (e.g. shipping) and will be frozen on sight, then, after dilution with TEYG, they are placed in a 37 °C water jacket and cooled to 5 °C over 2 h.

Then, load the samples into 0.5 mL CBS or wick and powder (aka French) semen straws.

Semen cryopreservation:

Samples can be frozen one of two ways:

Box freezing: Samples are placed on a rack and frozen in liquid nitrogen vapor (4.5 cm above liquid nitrogen) for 10 min and plunged into the liquid nitrogen for storage.

Programmable freezer: The samples are frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5 °C to -10 °C at 5 °C/min; -10 °C to -110°C at 40 °C/min; -110°C to -140°C at 20°C/min. Plunge into liquid nitrogen for storage.

Thawing: Thaw samples for 30 s in a 37 ºC water bath and evaluate motility as described previously.

Artificial insemination:
Following estrous synchronization, single semen straws are used per insemination. Either single or double (2 inseminations per estrus) inseminations may be performed.

Transportation:
Please see the Impact Shipper Protocol on the Animal GRIN webpage noting the specific temperatures for each species and type of tissue:
https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/animal/animal-protocols/

Recipes:

Tris-egg yolk-glycerol (TEYG) diluent, 100 mL volume**:**

Tris	2.422 g
Fructose	1.0 g
Citric Acid	1.36 g
Penicillin G	0.006 g
Streptomycin sulfate	0.100 g
Egg yolk	2.5% by volume
Glycerol	2.0 % by volume
pH to 6.8-7.0	

References:
Mook, J.L. Wildeus, S. 2008. Effect of egg yolk level, washing and extended pre-freeze equilibration on postthaw motility of buck semen. Southern Section American Society of Animal Science Annual Meeting. Dallas, TX.

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